Revista Chilena de Historia Natural
74:763-767, 2001

Cryptobia neghmei sp. n. (Protozoa: Kinetoplastida) in two species of flounder, *Paralichthys* spp. (Pisces: Paralichthydae) off Chile

Cryptobia neghmei sp. n (Protozoa: Kinetoplastida) en dos especies de lenguados *Paralichthys* spp. (Pisces: Paralichthydae) de la costa de Chile

RASUL A. KHAN[1], VÍCTOR LOBOS[2], FÉLIX GARCÍAS[2], GABRIELA MUÑOZ[2], VERÓNICA VALDEBENITO[2] & MARIO GEORGE-NASCIMENTO[2]

[1]Department of Biology, Memorial University of Newfoundland, St. John's, Newfoundland, Canada, A1B 3X9
[2]Facultad de Ciencias, Universidad Católica de la Santísima Concepción, Casilla 297, Concepción, Chile

ABSTRACT

Cryptobia neghmei sp.n. is described from the blood of two species of flounder, *Paralichthys microps* and *P. adspersus*, inhabiting the Chilean coast in the southern Pacific Ocean. Flagellates were elongate, slender, with two flagella and a conspicuous undulating membrane. It was distinguished from previously described species on the basis of its unusual shape and dimensions. All of 97 flounder were infected upon examination. Developmental stages of kinetoplastid protozoans, perhaps C. *neghmei* sp. n., were observed in some leeches *Glyptonotobdella* sp. that were found attached to flounder, which probably represent a mode for transmission among piscine hosts.

Key words: *Cryptobia neghmei*, hemoflagellate, flounder, *Paralichthys microps*, *Paralichthys adspersus*, coastal Chile.

RESUMEN

Se describe a *Cryptobia neghmei* sp.n., un protozoo sanguíneo de dos especies de lenguados, *Paralichthys microps* y *P. adspersus,* habitantes de la costa de Chile en el sur-este del océano Pacífico. Los protozoos flagelados son de forma elongada, delgados con dos flagelos y una membrana ondulante conspicua. Esta especie se distingue de aquellas descritas previamente en base a su forma y dimensiones inusuales. Los 97 lenguados revisados estaban infectados. Se observaron otros estados de desarrollo de protozoos kinetoplástidos en la sanguijuela *Glyptonotobdella* sp. que parasitaba a los lenguados y que probablemente sea el vector del protozoo.

Palabras clave: *Cryptobia neghmei*, hemoflagelado, lenguado, *Paralichthys microps*, *Paralichthys adspersus*, costa de Chile.

INTRODUCTION

Among kinetoplastid protozoans of fish, two morphologically similar genera (*Cryptobia* Leidy 1846 and *Trypanoplasma* Laveran and Mesnil 1901) are recognized mainly on the basis of their location on (or in) the host body. Their life cycles can involve direct transmission or by blood sucking leech species. Species of the genus *Cryptobia* are kinetoplastid flagellates which possess two free-moving flagella, one located anteriorly, the other one posteriorly attached, and invariably an undulating membrane extending the full length of the body. They have been reported from some invertebrates but more often in the blood of fish (Becker 1977, Khan 1991).

This study was conducted on the blood parasites of two species of flounder, *Paralichthys microps* (Günther 1881) and *P. adspersus* (Steindachner 1867) living off the coast of Chile. An ectoparasitic leech was also taken from some of the fish and examination of its intestinal contents revealed a number of developmental stages of kinetoplastid protozoans. Since morphometric criteria of this organism were not comparable to those of any species described previously, it is considered a new species. A description of the parasite in flounder and other developmental stages of kinetoplastid protozoans found in the leech are reported herein.

MATERIAL AND METHODS

Flounder were captured by gillnet and by otter trawl off the coast of Chile at three locations:

Concepción bay, San Vicente bay, and Gulf of Arauco, at depths 8-15 m during November and December, 1998 (Fig. 1). The fish were bled from the caudal artery after capture using a 3.0 ml syringe that was heparinised and 23 gauge needle. The fish varied in length from 20 to 55 cm and weighed 65 to 2,100 g. Smear made from living specimens were exposed to iodine vapour for about one minute to prevent contraction. Fresh smears in phosphate buffer (pH 7.6) from the gills and skin of these fish were also examined for parasites. Thick (one drop of blood) and thin smears were prepared from all other fish, air-dried, incubated at 60 °C for 30 min before fixation with methanol and staining with Giemsa (1:10 dilution with phosphate buffer, pH 7.6) for about one hour. Some smears were also stained with "diff quik" (Baxter Scientific Products, Illinois, U.S.A.). Three specimens of a leech, collected from the bodies of three flounder, were dissected and smears prepared on slides were stained with Giemsa. Ilustrations of the parasite were made from photomicrographs using a Zeiss "ultraphot" with a micrometer and measurement (mm) were made from stained smears. Means and standard errors were determined.

RESULTS

Description of the parasite is based on five living (which were also recorded on a video tape) and 25 stained (both Giemsa and "diff quik") specimens which were obtained from five hosts (5 per fish). None was seen in smears made from the surface (both dorsal and ventral) of the body or from the gills.

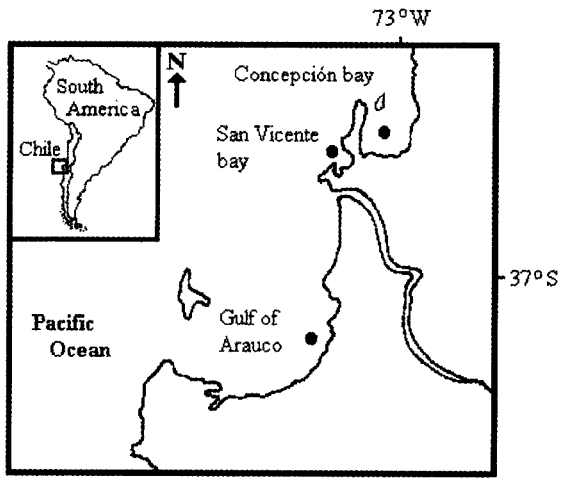

Fig. 1: Sampling locations (black circles) of the flounder *Paralichthys* spp. off the coast of Chile.

Localidades (círculos negros) de muestreo de los lenguados *Paralichthys* spp. frente a la costa de Chile.

Genus Cryptobia *Leidy 1845*

Cryptobia neghmei sp.n. (Fig. 2A, 2B, 2C). Diagnosis (blood stream forms, mean ± SE measurements in mm; based on living and Giemsa-stained specimens). Body elongate 41.9 ± 0.4 x 3.8 ± 0.6, narrow, tapering toward the posterior extremity of body flexing continually in live specimens; anterior flagellum prominent, moving rapidly, 10.3 ± 1.1 mm long; distinct undulating membrane extending the full length of the body (Fig. 2), beating rapidly in a wave-like manner, posteriad; posterior flagellum short, 5.2 ± 0.4 long, flicking but moving less rapidly than the anterior flagellum; nucleus ovoid to elongate, 3.8 ± 0.2 x 3.2 ± 0.1, located 6.8 ± 0.1 from anterior extremity, appearing translucent in living specimens and light gray when stained; in one stained specimen, an extension of the nucleus was observed, kinetoplast elongate, 9.7 ± 0.8 x 3.6 ± 0.2, magenta-stained at the anterior extremity, lying near the flagellum in stained specimens but ovoid and translucent in live specimens.

Kinetoplastid stages in the leech Glyptonotobdella *sp. Sawyer and White, 1969, an ectoparasite of flounder*

Several developmental stages of kinetoplatid protozoans were observed in stained smears prepared from the digestive tract of three specimens of a leech *Glyptonotobdella* sp., that were taken from the body surface of flounder. Some were ovoid, (11.6 ± 0.9 x 7.1 ± 0.4) with a nucleus (Fig. 3A), and kinetoplast and appeared to be diving with pale-stining flagella that were faintly visible (Fig. 3B). Others were ovoid to pyriform, nucleated with an anteriorly located kinetoplast and a flagellum 12.5 ± 1.1 x 8.6 ± 1.0 (Fig. 3C, 3D).

Taxonomic summary

Type host: *Paralichthys microps* and *P. adspersus.*

Site of infection in host: blood.

Geographical location: coast of Chile (near to 36° 44´S, 73° 09´W) in the South Pacific Ocean.

Prevalence: 100 % from 97 flounder examined.

Vector: probably the leech *Glyptonotobdella* sp., *G. antarctica* was reported in this area of Chile, as (misnamed) *Glyptonobdella* by Enríquez-Briones and Gallardo (1994) on the red shrimp *Pleuroncodes monodon* Milne Edwards, 1837 (Anomura: Galatheidae).

Etymology: the species is named after Professor Amador Neghme in recognition of his contributions to parasitology in Chile.

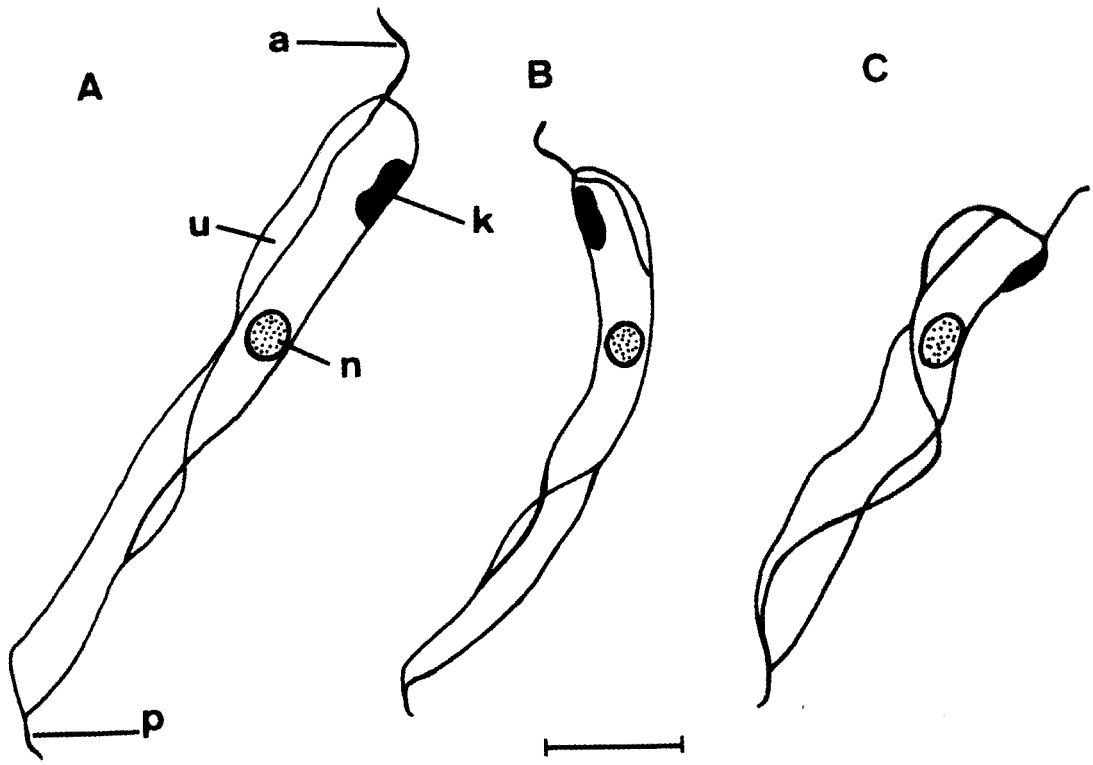

Fig. 2: (A), (B), (C) Morphotypes of *Cryptobia neghmei* sp. n. from the blood of *Paralichthys microps* and *P. adspersus*. Scale bar is 10 µm. a: anterior flagellum, k: kinetoplast, n: nucleus, p: posterior flagellum, u: undulating membrane.

Morfotipos de *Cryptobia neghmei* sp. n. de la sangre de *Paralichthys microps* y *P. adspersus*. Línea de cota es de 10 µm. a: flagelo anterior, k: kinetoplasto, n: núcleo, p: flagelo posterior, u: membrana ondulante.

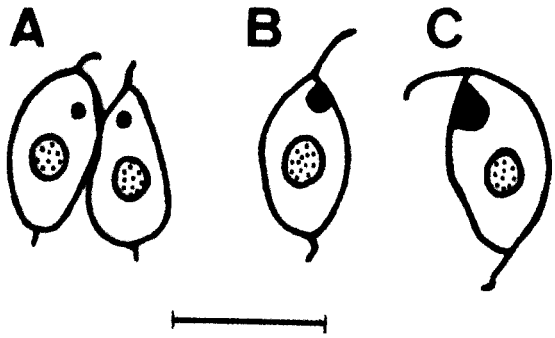

Fig. 3: (A), (B), (C) Intermediate developmental stages of kinetoplastid protozoans from the leech *Glyptonotobdella* sp. Scale bar is 10 µm.

Estadios de desarrollo intermedio de protozoos kinetoplástidos de la sanguijuela *Glyptonotobdella* sp. Línea de cota es de 10 µm.

Etymology: the species is named after Professor Amador Neghme in recognition of his contributions to parasitology in Chile.

Specimens: Holotype: deposited in the Museo Nacional de Historia Natural, Santiago, Chile; Catalogue number: MNHN-PR-N° 3006; Paratypes, catalogue number: MNHN-PR-N° 3007. Voucher specimens were deposited in U.S. National Parasite Collection USNPC N° 091502.00.

DISCUSSION

Two types of kinetoplastid flagellates are recognized in *Paralichthys* (see Sypek & Burreson 1983, Khan 1991): *Cryptobia* and *Trypanoplasma* are morphologically similar. Based on their location, an ectocomensal group is referred to as species of *Cryptobia* and another living in the blood stream as *Trypanoplasma*. Transmission of the former is direct (host to host) without other developmental changes whereas the latter are transmitted by hematophagous leeches in which a

number of development stages occur (Burreson 1979b, 1982). However, a review by Woo (1984), with which we concur, considered the genus *Trypanoplasma* a synonym of *Cryptobia*, based not only on the morphological similarity of the genera but also on observations that at least one hematozoic species could be transmitted both directly and by a blood-sucking leech. Recent molecular evidence revealed that *T. borreli* was more closely related to *C. salmositica* than to *C. bullocki* or *C. catostomi*, raising doubts about the validity of the genus *Trypanoplasma* (Wright et al. 1999). However, Lom & Dykova (1992) continue to use *Trypanoplasma* for biflagellated haematozoans transmitted by leeches.

Cryptobia neghmei sp. n. can be distinguished from other described species on account of its unusual morphology and dimensions. While it appears similar to *Cryptobia* (= *Trypanoplasma*) *beckeri*, it is about half of the total length of the latter [mean: 109 (43-165) µm] species (Burreson 1979a). A species of *Cryptobia* was observed in the blood of a flounder, *Limanda aspera*, in the North Pacific Ocean adjacent to British Columbia, but no description of the parasite was made (Sloan et al. 1984). Since 90 % of the piscicolid leech, *Notostomum cyclostomum* were infected, it was suggested this was probably the vector. A similar parasite was described from an Antarctic eelpout, *Rhigophila dearborni*, but only one flagellum was observed (Becker & Halloway 1968). Subsequent examination of the parasite by Burreson (1979a) revealed a recurrent flagellum, confirming its biflagellated status. We concur with Burreson (1979a) that in air-dried blood films, the recurrent flagellum might not be detected unless living specimens are examined.

The life cycle of *C. neghmei* sp. n. might be similar to that of *C.* (= *T.*) *beckeri* if developmental stages observed in the piscicolid leech, *Glyptonotobdella* sp. prove to be conspecific to *C. neghmei*. Burreson (1979a) demonstrated that flagellates ingested by the leech, *Malmiana diminuta*, became rounded and underwent binary fission in the crop. Eventually, small infective stages entered the proboscis sheath and were transmitted following the next blood meal of the leech. Burreson (1979a, 1979b) suggested that growth of the infective forms in the piscine host was rapid as only large individuals were observed 8 to 9 days after transmission in laboratory experiments. The absence of any small forms in the *Paralichthys* spp. in the present study, provides support for this view. Woo (1987) noted that at least two hematozoic species also had ectoparasitic stages and one was transmitted directly via water (see also Brower & Margolis 1983, Woo & Wehnert 1983). One of these included *C. bullocki* which infects several species of flatfish inhabiting the eastern seaboard of North America (Burreson & Zwerner 1982). Although we did not observe *C. neghmei* on the gill or skin of *Paralichthys* spp., it is likely that the infections were too low to detect. Further studies should focus on direct transmission, especially in juvenile flounder which tend to exhibit higher parasitemias than adults (Burreson & Zwerner 1984). Moreover, a knowledge of the life cycle and pathogenicity of *C. neghmei* is relevant since there is evidence that *C. bullocki*, causes morbidity and mortality in juvenile summer flounder, *Paralichthys dentatus*, both in the laboratory and in the field (Burreson & Zwerner 1982, 1984). The fish were anemic had abdominal distension with ascites and splenomegaly specially when the water temperature was low (Sypek & Burreson 1983). Although anemia was observed in the infected flounder, this condition might have been the result of synergism between *C. neghmei* sp. n. and exposure to a variety of contaminants (George-Nascimento et al. 2000). Further studies on juvenile flounder inhabiting uncontaminated areas along coastal Chile coupled with experimental infections in the laboratory might reveal the impact of this parasite on fish health. Additional studies should focus on its mode of transmission since at least one species, *C. salmositica* can be transmitted by a leech and directly via seawater.

ACKNOWLEDGMENTS

The authors are grateful for financial support from FONDECYT 1980442 and FONDAP Program 3, Marine Biology and Conservation (to MG-N), the Natural Sciences and Engineering Council of Canada (to RAK) and to Ms. S. Wall for typing the manuscript.

LITERATURE CITED

BECKER CD & HL HALLOWAY (1968) A survey of haematozoa in Antarctic vertebrates. Transactions of the American Microscopical Society 87: 354-360.

BECKER CD & M KATZ (1965) Transmission of the hemoflagellate, *Cryptobia salmositica* Katz, 1951, by a rhynchobdellid vector. Journal of Parasitology 51: 95-99.

BECKER CD (1977) Flagellate parasites of fish. In: Kreier JP (ed) Parasitic protozoa: 357-416. Academic Press, New York, New York.

BURRESON EM (1979a) Structure and life cycle of *Trypanoplasma beckeri* sp. n. (Kinetoplastida), a parasite of the cabezon, *Scorpaenichthys marmoratus*, in Oregon coastal waters. Journal of Protozoology 26: 343-347.

BURRESON EM (1979b) The life cycle of *Trypanoplasma bullocki* (Zoomastigophorea: Kinetoplastida). Journal of Protozoology 29: 72-77.

BURRESON EM & JP SYPEK (1981) *Cryptobia* sp. (Mastigophorea: Kinetoplastida) from the gills of marine fishes in the Chesapeake Bay. Journal of Fish Disease 4: 519-522.

BURRESON EM & DE ZWERNER (1982) The role of host biology, vector biology, and temperature in the distribution of *Trypanoplasma bullocki* infections in the lower Chesapeake Bay. Journal of Parasitology 68: 306-313.

BURRESON EM & DE ZWERNER (1984) Juvenile summer flounder, *Paralichthys dentatus*, mortalities in western Atlantic Ocean caused by the hemoflagellate *Trypanoplasma bullocki*: evidence from field and experimental studies. Helgolander Meeresunter-Suchungen 37: 343-352.

ENRÍQUEZ-BRIONES S & VA GALLARDO (1994) *Glyptonobdella antarctica* (Sawyer & White, 1969) (Hirudinea: Piscicolidae) epizoo ocasional del langostino colorado *Pleuroncodes monodon* H. Milne Edwards, 1837 (Anomura: Galatheidae). Gayana Oceanología (Chile) 2: 1-3.

GEORGE-NASCIMENTO M, RA KHAN, F GARCÍAS, V LOBOS, G MUÑOZ & V VALDEBENITO (2000) Impaired health in flounder, *Paralichthys* spp. inhabiting coastal Chile. Bulletin of Environmental Contamination and Toxicology 64: 184-190.

KHAN RA (1991) Further observations on *Cryptobia dahli* (Mastigophora: Kinetoplastida) parasitizing marine fish. Journal of Protozoology 38: 326-329.

LOM J & I DYKOVA (1992) Protozoan parasites of fishes. Elsevier, Amsterdam, The Netherlands. 315 pp.

SYPEK JP & EM BURRESON (1983) Influence of temperature on the immune response of juvenile flounder, *Paralichthys dentatus*, and its role in the elimination of *Trypanoplasma bullocki* infections. Developmental and Comparative Immunology 7: 277-286.

WOO PTK (1987) *Cryptobia* and cryptobiosis in fishes. Advances in Parasitology 26: 199-237.

WOO PTK & SD WEHNERT (1983) Direct transmission of a hemoflagellate, *Cryptobia salmositica* (Kinetoplastida: Bodonina) between rainbow trout under laboratory conditions. Journal of Protozoology 30: 334-337.

WRIGHT ADG, S LI, SJ FENG, SD MARTIN & DH LYNN (1999) Phylogenic position of the kinetoplastids, *Cryptobia bullocki, Cryptobia catostomi*, and *Cryptobia salmositica* and monophyly of the genus *Trypanosoma* inferred from small subunit ribosomal RNA sequences. Molecular Biochemical Parasitology 99: 69-76.

Associate Editor: P. Ojeda
Received December 11, 2000; accepted June 28, 2001